In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 4, lines 14-22 and replace it with the following paragraph:

In another aspect of the present invention, there is provided an isolated corepressor polypeptide having within its amino acid sequence at least two C-terminal binding protein interaction motifs, the first C-terminal binding protein interaction motif comprising the sequence PLDLTVR **(SEQ ID NO: 15)**, and the second C-terminal binding protein interaction motif comprising the sequence VLDLSTK **(SEQ ID NO: 16)**. The corepressor polypeptide is operably interactable with a C-terminal binding protein (CtBP) corepressor in a pathway to repress expression of DNA. In one embodiment, the isolated polypeptide comprises the amino acid sequence as set forth in Fig. 1D (SEQ ID NO:2).

Please delete the paragraph on page7, lines 31-32 and replace it with the following paragraph:

Figs. 1A-D illustrate the LCoR corepressor gene (SEQ ID NO: 1), transcript (SEQ ID NO: 3) and protein structure (SEQ ID NO: 2**; as well as SEQ ID NOS 19 and 23-30**).

Please delete the paragraph on page 8, lines 8-16 and replace it with the following paragraph:

Figs. 3A-C illustrate the interaction of LCoR and ER α *in vivo*. Fig. 3A illustrates Western analysis of LCoR in 20, 50 or 100 μg of extract from MCF-7, HEK293 and COS-7 cells using a rabbit polyclonal antipeptide antibody. Fig. 3B illustrates coimmunoprecipitation of LCoR with ER α. Fig. 3C illustrates bioluminescence resonance energy transfer (BRET) assays on COS-7 cells transiently cotransfected with plasmids

expressing EYFP-ER α and rluc-LCoR or rluc-LCoR-LSKAA **(SEQ ID NO: 17)** fusion proteins and treated with 10^{-7}M β-estradiol (E2), hydroxytamoxifen (OHT), raloxifene, diethylstilbestrol (DES) or ethanol (-).

Please delete the paragraph on page 8, lines 22-23 and replace it with the following paragraph:

Figs. 7A-7G illustrate that LCoR interacts with C-terminal binding proteins **(SEQ ID NOS 15 and 16)**.

Please delete the paragraph on page 13, lines 5-28 and replace it with the following paragraph:

LCoR was found to interact with the corepressor CtBP1 through tandem consensus CtBP-interaction motifs. Like LCoR, the sensitivity of repression by CtBPs to TSA is dependent on the promoter tested, indicative of HDAC-dependent and -independent modes of action (Chinnadurai, G. (2002) Mol. Cell, 9, 213-24). CtBP proteins interact with several different transcriptional repressors (Chinnadurai, G. (2002) Mol. Cell, 9, 213-24), including the nuclear receptor corepressor RIP140. The TSA-sensitive and -insensitive actions of LCoR are analogous to another CtBP-interacting repressor Ikaros, which is composed of distinct domains mediating repression by HDAC-dependent and -independent mechanisms. CtBP binding to Ikaros contributes to its HDAC-independent mode of action. CtBPs also associate with specific polycomb group (PcG) repressor complexes, and HDAC-independent repression of transcription by CtBP has been linked to its association with PcG complexes (Dahlya, A. et al. (2001) Mol. Cell, 8, 557-68). The present experiments indicate that LCoR also associates with components of PcG complexes. Therefore, in accordance with another aspect of the present invention there is provided an isolated corepressor polypeptide having within its amino acid sequence at least two C-terminal binding protein interaction motifs, said first C terminal binding protein interaction motif comprising the sequence PLDLTVR **(SEQ ID NO: 15)**, and said second C-terminal binding protein interaction motif comprising the sequence

VLDLSTK **(SEQ ID NO: 16)**, said polypeptide operably interactable with a C-terminal binding protein (CtBP) corepressor in a pathway to repress expression of DNA.

Please delete the paragraph on page 14, lines 7-22 and replace it with the following paragraph:

The action of corepressors such as LCoR that recognize agonist-bound receptors indicates that there are signals that act to attenuate the consequences of hormone-induced receptor function. Such effects would provide a counterbalance to signals that augment hormone- induced transactivation; for example the stimulatory effects of MAP kinase signaling on ERoc function (Kato, S. et al. (1995) Science, 270, 1491-4). Because LCoR acts to attenuate the function of agonist-bound receptors, posttranslational modification or LCoR and/or receptors will affect the relative affinities of LCoR and p160s for coactivator binding pockets. LCoR contains several putative phosphorylation motifs, including a number of MAP kinase sites in the region of the NR box, as well as potential sites for protein kineses A and C. Thus, LCoR's interaction with ligand- bound nuclear receptors can be modulated by phosphorylation. In addition, LCoR contains a consensus leptomycin B-sensitive nuclear export signal ($LX_3LX_3LXIX_3L$, a.a.149-164)**(SEQ ID NO: 18)**, indicating that its access to receptors is regulated by nuclear export under some conditions.

Please delete the paragraph on page 19, line 15, to page 20, line 29 and replace it with the following paragraph:

GST fusions in pGEX2T of ERa-LBD, TIF-2.1, and hVDR-LBD, HG1, hPR, ERE3-TATA/pXP2, 17mer5-tk/pXP2, GAL4-DBD(1-147)/pSG5, TIF-2.1/pSG5, TIF2/pSG5 have been described (Aumais, J. et al. (1996) J. Biol. Chem., 271, 12568- 12577; Lee, H.S. et al. (1996) J. Biol. Chem. 271, 25727-25730; Eng, F.C.S. et al. (1998) J. Biol. Chem., 273, 28371-7).! ERa-mAF2 was constructed by point mutagenesis of L539 and L540 to A residues. ERa-EYFP was constructed by insertion of an ERa cDNA lacking a stop codon into EcoRI and BamHI sites of pEYFP-CMV. For ER378/pSG5, a.a 1-378 of ERa was amplified using 5' primer I 5'CCGGMTTCCGGATGACCATGACCCTCCAC3' (SEQ ID NO:5) and 3'

25 primer 5'CGGGATCCCGTCAAAGGTGGACCTGATCATG3' (SEQ ID NO:6) and subcloned in EcoRI/BamHI digested pSG5. The GRE5 promoter was excised with Xbal and BamHI and subcloned to the Smal/Bgill sites of pXP2 to make GRE5/pXP2, and VDRE3tkCAT was digested with BamHI and Bgill and VDRE3tk subcloned into pXP2 to give VDRE3tk/pXP2. ERa mutants T347A, N359S, and H356R were identified by sequencing of clones of the LBD mutagenized by PCR amplification. Mutagenized LBD sequences were subcloned as Hindlil-Xbal fragments into Hindlil-Xbal digested pGEX2T-ERa-LBD. The 475- 918bp region of LCoR was amplified I with 5' primer 5'CCGGMTTCCGGCCCGGGCATGAGACAGTCCCTG - 20 5 GGTCTC3' (SEQ ID NO:7) and a 3' primer with an endogenous Kpnl site (position 918bp) 5'TTCTTGGAGGTACCCCATCA3' (SEQ ID NO:8) and inserted into 918-2915 LCoR/pSG5 digested with EcoRI and Kpnl to create 475-2915 LCoR, which contains a full-length ORE (subsequently called; LCoR/pSG5), and into pGEM-T-easy (Promega, Madison, Wl) to create I 10 probes for in situ hybridization. The PCR fragment was verified by sequencing. LCoR/pSG5 was digested with Sfrl and BamH1 and subcloned in BamHI site of GAL4DBD/pSG5 to create GAL4-LCoR/pSG5. Point mutagenesis of LSKLL **(SEQ ID NO: 19)** to LSKAA **(SEQ ID NO: 17)** at position 53, and deletion of PLDLTVR **(SEQ ID NO: 15)** (a.a. 64-70; m1) and VLDLSTK **(SEQ ID NO: 16)**(a.a. 82-88; m2) were made by QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). For GST-LCoR and GST-LSKAA **(SEQ ID NO: 17)**, PCR amplification of LCoR or LCoR-LSKAA **(SEQ ID NO: 17)** was performed with 5'CGCGGATCCGCGATGCAGCGMTGATCCM3' (SEQ ID NO:9), and 5'GGMTTCCCTACTCGTTTTTTGATTCATT3' (SEQ ID NO:10), digested with BamHI and EcoRI, and inserted into pGEX2TK. For LCoR-rluc, LCoR or LCoR-LSKAA **(SEQ ID NO: 17)** were amplified with 5' primer 5'CTAGCTAGCCACCATGCAGCGMTGATCCAA3' (SEQ ID NO:11) and 3' primer 5'CTAGCTAGCCGCTCGTTTTTTGATTCATT3' (SEQ ID NO:12). PCR products were digested with Nhe1 and cloned into pRL-CMV (Promega), and verified by sequencing. For HA-LCoR, HA-LSKAA **(SEQ ID NO: 17)**, Flag 25 LCoR and Flag- LSKAA **(SEQ ID NO: 17)**, cDNA sequences from LCoR/pSG5 or LSKAA/pSG5 **(SEQ ID NO: 17)** were amplified using 5'CGGAATTCCAGCGAATGA TCCMCM3' (SEQ ID NO:13) and 5'CGCGGATCCGCGCTACTCG TTTTTTGATTCATT3' (SEQ ID NO:14), digested with

EcoRI and BamHI and inserted into the corresponding sites of HA/pCDNA3 or Flag/pCDNA3.

Please delete the paragraph on page 20, line 31, to page 21, line 10 and replace it with the following paragraph:

All cell lines were cultured under the recommended conditions. COS-7 cells grown in 6-mm plates in DMEM without phenol red, supplemented with 10% FBS were transfected in medium without serum with lipofectamine 2000 (Invitrogen, Burlington, Ont.) with 100ng of nuclear receptor expression vectors as indicated, 200ng of TIF-2 or TIF-2.1, as indicated, 250ng of reporter plasmid, 250ng of internal control vector pCMV-,Bgal, and various concentrations of LCoR/pSG5 or LCoR LSKAA/pSG5 **(SEQ ID NO: 17)** expression vectors and pSG5 carrier. Medium was replaced 24h after transfection by a medium containing charcoal-stripped serum and ligand (100nM) and TSA (AM) for 18h, as indicated. Cells were harvested in 200 ul of reporter lysis buffer (Promega).

Please delete the paragraph on page 22, line 26, to page 23, line 11 and replace it with the following paragraph:

LCoR of Fig. 1 bears only limited resemblance to known coregulators. There is a single LXXLL motif (NR box) at amino acid 53, and a PRKKRGR **(SEQ ID NO: 20)** motif at amino acid 339 that is homologous to a simple nuclear localization signal (NLS) of the SV40 large T antigen-type. The NLS lies at the N-terminus of a putative helix-loop-helix domain (Figs. 1C and D, SEQ ID NO:1-3), which is 48, 48, and 43% homologous to motifs encoded by the Eip93F, T01C1.3, and MBLK-1 genes of Drosophila, C elegans, and Honeybee (Apis mellifera), respectively (Fig. 1C; SEQ ID · NO:3). The domain also bears 35% homology to the pipsqueak motif (PSQ) repeated four times in the DNA binding domain of the Drosophila
transcription factor pipsqueak. Fig. 1C is a schematic representation of an LCoR compressor protein of the present invention. The NR box LSKLL **(SEQ ID NO: 19)**, nuclear localization signal (NLS), and putative helix- loop-helix (HLH) domain are indicated. The homologies of

the HLH with other proteins are shown, with asterisks indicating positions of amino acid similarity. Existence of the HLH was predicted using Psired (http://bioinf. cs.ucl. ac.uk) and Network Protein Sequence Analysis (http://pbil.ibcp.fr).

Please delete the paragraph on page 23 lines 12-15 and replace it with the following paragraph:

In Fig.1D, the sequence of 1826bp of a LCoR cDNA (SEQ ID NO:1) and complete predicted 433 amino acid protein (SEQ ID NO:2) sequences are presented. The LSKLL **(SEQ ID NO: 19)** is boxed, the NLS is underlined, and the helix loop-helix domain is highlighted.

Please delete the paragraph on page 25, lines 13-30 and replace it with the following paragraph:

Interaction of ERα and LCoR in vivo was further tested by bioluminescence resonance energy transfer (BRET) in living COS-7 cells transiently cotransfected with plasmids expressing ERα-EYFP and LCoR-rluc fusion proteins. Consistent with coimmunoprecipitations, treatment with estradiol or diethylstilbestrol (DES) enhanced BRET ratios 2.5 to 3- fold (Fig. 3C), consistent with agonist-dependent interaction of LCoR and ERα, I whereas treatment with antiestrogens 4- hydroxytamoxifen (OHT) or raloxifene had no significant effect. Fig. 3C illustrates Bioluminescence resonance energy transfer (BRET) assays on COS-7 cells transiently cotransfected with plasmids expressing EYFP-ERα and rluc- LCoR or rluc-LCoR-LSKAA **(SEQ ID NO: 17)** fusion proteins and treated with 1 0-7M,8-estradiol (E2), hydroxytamoxifen (OHT), raloxifene, diethylstilbestrol (DES) or ethanol (-). BRET ratios were calculated as described in experimental procedures. The data shown represent the mean +SEM of 3 experiments. Moreover, mutation of the NR box of LCoR to LSKAA **(SEQ ID NO: 17)** largely disrupted hormone-dependent interaction and reduced hormone-independent interaction of the two proteins by approximately two-fold (Fig. 3C), indicating that the LCoR LXXLL motif is essential for ligand-dependent interaction with ERα.

Please delete the paragraph on page 26, lines 8-24 and replace it with the following paragraph:

In vitro translated LCoR selectively bound to the ERα LBD fused to GST (GST-ERα-LBD) in a partially estrogen-dependent manner (Fig. 4A). In Fig. 4, Estradiol (E2), hydroxytamoxifen (OHT), raloxifene (Ral), and ICI164,384 (ICI), vitamin D3 (D3) were added to 10^{-6} M as indicated. Inputs (lanes 1) represent 10% of the amount of labeled protein used in assays. Fig. 4A illustrates ligand-dependent interaction of *in vitro*-translated LCoR with GST-ERα LBD. Figs. 4B and 4D illustrate the interaction of in vitro translated ERα (HEG0; B) or ER378 (D) with GST fused to LCoR, LCoR LSKAA **(SEQ ID NO: 17)** or TIF2.1 as indicated. Fig. 4C illustrates the interaction of LCoR with GST-ERα or a helix 12 mutant (ERα -mAF-2). Figs. 4E and 4F illustrate the interaction of GST fusions of wild-type ERα LBD or LBD mutants T347A, H356R, N359S, and K362A with LCoR (E) or TIF-2.1 (F). Histograms of results of triplicate experiments are shown. Bands were quantitated using the FluorChem digital imaging system and AlphaEaseFC software (Alpha Innotech Corp, San Leandro, CA). Figs. 4G and 4H illustrate the Interaction of ERβ (G) and VDR (H) with GST-LCoR and GST-LSKAA **(SEQ ID NO: 17)**.

Please delete the paragraph on page 26, line 25 to page 27, line 7 and replace it with the following paragraph:

Consistent with BRET analyses, antiestrogens OHT, raloxifene, or ICI 164,384 did not induce interaction of LCoR with ERα (Fig. 4A), and hormone-dependent binding of ERα was abolished by mutation of the LCoR NR box (LSKAA **(SEQ ID NO: 17)**; Fig. 4B). Similar results were obtained with GST ERα fusions and *in vitro* translated LCoR-LSKAA **(SEQ ID NO: 17)**. Furthermore, double point mutation of the ERoc AF-2 domain in helix 12 (L539A, L540A; mAF-2) abolished ligand-dependent binding of LCoR (Fig. 4C). ERα was truncated to amino acid 378 (ER378), leaving regions A-D and the N-terminal third of the LBD (Fig. 4D), or to amino acid 282 in region D (HE15) or 180, which encodes the A/B domain. While ER378 bound specifically to GST-LCoR, but not TIF-2.1, in a hormone-independent manner (Fig. 4D), no such interaction was observed with HE15 or the Ai'B

domain, suggesting that residues contributing to ligand-independent interaction with LCoR are located between ERα amino acids 283 and 377.

Please delete the paragraph on page 27, line 31 to page 28, line 22 and replace it with the following paragraph:

The effects of LCoR on transactivation by nuclear receptors were tested by transient transfection in COS-7 cells (Fig. 5), which revealed that LCoR is a repressor of ligand-dependent transcription of class I and 11 receptors. In Figs. 5A, 5C, 5D, OF, and 5H, LCoR represses ERα-, GR-, PR- and VDR-dependent transactivation. COS-7 cells were cotransfected with expression vectors for ERα HEGO (A and C) or GR (D) or PR (F) or VDR (H), ERE3-TATA-pXP2 (A and C), GRE5/pXP2 (D and F) or VDRE3tk/pXP2 (H) luciferase reporter vectors, pCMV--gal as internal control, and LCoR/pSG5 or LSKAA/pSG5 **(SEQ ID NO: 17)** expression vectors as indicated. Cells were treated with 10^{-7}M of hormones (solid bars) or vehicle (open bars). Normalized luciferase activities (RLU) are the means ± SEM from at least 3 experiments. The inset of Figure 5A illustrates control western blot of ERα from extracts of COS-7 cells transfected with ERα HEGO and 0, 500 or 1000ng of LCoR/pSG5 in the absence or presence of estradiol. Fig. 5C illustrates that LCoR represses TIF-2 coactivation of ERα. Cells were transfected as in Fig. 5A with LCoR, TIF-2 or TIF2.1 as indicated. Fig. 5J illustrates a GAL4-LCoR fusion protein represses transactivation. COS-7 cells were transfected with 750ng of 1 7mer5tk/pXp2, with indicated amounts of GAL4-LCoR/pSG5 or 1000 ng of pSG5 or GAL4/pSG5. Normalized luciferase activities (RLU) are the means ± SEM from at least 3 experiments. Figs. 5B, 5E, 5G, 5I and 5K illustrate differing effects of HDAC inhibitor TSA on repression by LCoR. Transfections were performed as in the left-hand panels except that TSA (3 uM) was added.

Please delete the paragraph on page 28, line 23 to page 29, line 14 and replace it with the following paragraph:

Coexpression of LCoR produced a dose-dependent repression of hormone- dependent transactivation by ERα, which was abolished by mutation of the NR box, as the LSKAA **(SEQ ID NO: 17)** mutant had no effect on ERα function (Fig. 5A). Repression of estrogen-dependent gene expression was not due to downregulation of ERα protein in cells cotransfected with LCoR (Fig. 5A, inset). Similar results were obtained in MCF-7 and HEK293 cells. Consistent with LCoR and TIF-2 recognizing overlapping binding sites on ERα, LCoR repressed estrogen-dependent expression coactivated by TIF2 or TIF2.1 (Fig. 5C). Repressive effects of 1,ug of transfected LCoR on ligand-activated transcription on the order of 2.2-5- fold were observed in experiments with the glucocorticoid, progesterone and vitamin D receptors, (Figs. 5D, F and H). In each case, mutation of the NR box disrupted transcriptional repression. Moreover, a GAL4-LCoR fusion repressed the activity of the 5x17mer-tk promoter in a dose-dependent manner by 4- fold (Fig. 5J), whereas as free LCoR had no effect on the 5x17mer-tk promoter. The mechanism of action of LCoR was investigated by analyzing the effect of the HDAC inhibitor trichostatin A (TSA) on repression of ligand-dependent transcription. Remarkably, while TSA completely abolished LCoR-dependent repression of ERα and GR function (Figs. 7B and E), it had little or no effect on repression of PR or VDR function, or on repression by GAL-LCoR (Figs. 5G, I and K), indicating that LCoR may function by HDAC-dependent and independent mechanisms.

Please delete the paragraph on page 29, lines 17-20 and replace it with the following paragraph:

Pull-down assays performed with GST-LCoR and GST-LSKAA **(SEQ ID NO: 17)** to screen for potential interactions with class I HDACs 1 and 3, and class 11 HDACs 4 and 6 revealed that both LCoR proteins interacted with HDACs 3 and 6, but not with HDACs 1 and 4 (Fig. 6A).

Please delete the paragraph on page 29, line 21to page 30, line 9 and replace it with the following paragraph:

In Fig. 6A, HDACs 1, 3, 4, and 6 were *in vitro* translated and! incubated with GST alone or with GST-LCoR or GST- LSKAA **(SEQ ID NO: 17)** fusion proteins. The input (lane 1) represents 10% of the amount of labeled protein used in assays. Fig. 6B illustrates the association of tagged LCoR or LCoR-LSKAA **(SEQ ID NO: 17)** with HDAC3. Lysates from COS-7 cells transiently! transfected with HA-HDAC3 and Flag-LCoR or Flag- LSKAA **(SEQ ID NO: 17)**, were precipitated with anti-Flag antibody. Cell extract and immunocomplexes were analyzed by Western blotting with anti-HDAC3 or anti- Flag. Fig. 6C illustrates endogenous LCoR coimmunoprecipitates with endogenous HDAC3. Immunoprecipitations from MCF-7 cell extracts were performed with either rabbit control IgG or anti-HDAC3 antibody, and immunoprecipitates were probed for HDAC3 or LCoR as indicated. Fig. 6D illustrates association of LCoR and LCoR-LSKAA **(SEQ ID NO: 17)** with HDAC6. Lysates from COS-7 cells transiently cotransfected with HA-Flag-HDAC6 and HA 35 LCoR or HA-LSKAA **(SEQ ID NO: 17)**, were precipitated with anti-Flag antibody and the immunocomplexes were analyzed by Western blotting with anti- HA or anti-Flag. Fig. BE illustrates endogenous LCoR coimmunoprecipitates with endogenous HDAC6. Immunoprecipitations from MCF-7 cell extracts were performed with either rabbit control IgG or anti-HDAC6 antibody, and immunoprecipitates were probed for HDAC6 or LCoR as indicated.

Please delete the paragraph on page 30, lines 10-20 and replace it with the following paragraph:

Reciprocal coimmunoprecipitation experiments revealed an interaction between epitope-tagged LCoR or LCoR-LSKAA **(SEQ ID NO: 17)** and HDAC3 (Fig. 6B). Moreover, interaction between endogenous LCoR and HDAC3 was confirmed by coimmunoprecipitation with an anti-HDAC3 antibody from extracts of MCF-7 cells (Fig. 6C). Identical results were obtained in 15 extracts of HEK293 cells. Similarly, HA-LCoR and HA-LCoR-LSKAA **(SEQ ID NO: 17)** were coimmunoprecipitated with HA-Flag-HDAC6 by an anti-Flag antibody (Fig. 6D), and endogenous LCoR coimmunoprecipitated with HDAC6

from extracts of MCF-7 cells (Fig. BE). Taken together, these results indicate that LCoR can function to couple specific HDACs to ligand-activated 20 nuclear receptors.

Please delete the paragraph on page 30, line 23 to page 31, line 10 and replace it with the following paragraph:

Figs. 7A-7G illustrates that LCoR interacts with C-terminal binding proteins. Fig. 7A is a schematic representation of LCoR showing CtBP binding sites 1 and 2, and the position of the Mfe1 site used to create C-terminally truncated LCoR. In Fig. 7B, GST pull-down assays were performed with *in vitro* translated CtBP1, and GST control (pGEX) or fusions with LCoR, LCoR-LSKAA **(SEQ ID NO: 17)** or LCoR-Mfe1 deletion mutant. In Fig. 7C, GST pull-down assays were performed with *in vitro* translated CtBP1, and GST control (pGEX) or fusions with LCoR, LCoR-LSKAA **(SEQ ID NO: 17)** or LCoR mutated in CtBP binding sites 1 (m1), 2(m2) or 1 and 2 (m1+2). All GST fusion proteins were expressed at similar levels. Fig. 7D illustrates that LCoR coimmunoprecipitates with CtBPs. Extracts of MCF-7 cells were immunoprecipitated with rabbit control IgG or with a rabbit polyclonal anti-CtBP antibody, and immunoprecipitates were probed for CtBP1, CtBP2 or LCoR. Figs. 7E and 7F illustrate colocalization of LCoR and CtBP1 (E) or CtBP2 (F) by confocal microscopy. In Fig. 7G, mutation of CtBP binding motifs attenuates repression by LCoR. COS-7 cells were cotransfected with expression vectors for ERα or GR or PR as indicated, along with ERE3-TATA-pXP2 or GRE5/pXP2 as appropriate, and either wild-type LCoR or LCoR mutated in CtBP binding motifs 1 or 2 as indicated.

Please delete the paragraph on page 31, lines 11-22 and replace it with the following paragraph:

Analysis of LCoR sequence (Fig. 7A) revealed PLDLTVR **(SEQ ID NO: 15)** (a.a. 64) and VLDLSTK **(SEQ ID NO: 16)** (a.a 82) motifs that are homologous to the PLDLS/TXR/K **(SEQ ID NO: 21)** sequence defined as a binding site for the corepressor CtBP1. CtBP1, which was originally found as a protein that interacts with the C- terminus of 15 ETA, functions by HDAC-dependent and -independent mechanisms (Chinnadurai, G. (2002) *Mol.*

Cell, 9, 213-24), and is highly homologous to CtBP2. GST pull-down assays revealed an interaction between CtBP1 and wild-type LCoR, the LSKAA **(SEQ ID NO: 17)** mutant, and an LCoR mutant lacking the C- terminal half of the protein (LCoR-Mfe1). CtBP1 binding was abolished only 20 when both binding sites in LCoR were mutated (m1+2; Fig. 7C). While NADH can modulate CtBP function, no effect of NADH was seen on its interaction with LCoR *in vitro*.

Please delete the paragraph on page 33, lines 9-24 and replace it with the following paragraph:

Antibodies. A rabbit polyclonal antipeptide antibody was raised against LCoR a.a 20-36 (QDPSQPNSTKNQSLPKA) **(SEQ ID NO: 4)** fused to keyhole limpet hemocyanin, and purified over a peptide affinity column (Bethyi Laboratories, Montgomery TX). Rabbit polyclonal α-CtBP (sc-11390), goat polyclonal α-CtBP1 (sc-5963), goat polyclonal α-CtBP2 (sc-5967), goat polyclonal α- CtlP (sc-5970), goat polyclonal α-Rb (sc-1538), goat polyclonal α-Bmi1 (sc-8906), rabbit polyclonal α-Bmi1 (sc-10745), goat polyclonal HDAC3 (sc-8138), goat polyclonal HDAC6 (sc-5253), protein A agarose and protein A+G-agarose were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cy3-donkey polyclonal α-goat (705- 165-147) and Cy2-goat polyclonal α-rabbit (711 -225-152), Cy3-donkey polyclonal α 20 rabbit (711 -165- 152), Cy2-donkey polyclonal α-mouse (715-225-150) were purchased from Jackson ImmunoResearch (West Grove, PA, USA). Mouse monoclonal α-Flag M2 (F3165), and α-FLAG M2 HRP-conjugate (A-8592), monoclonal α-rabbit HRP conjugate (A2074), rabbit polyclonal α-goat HRP conjugate (A5420) were from Sigma (St. Louis, MO).

Please delete the paragraph on page 33, lines 25-31 and replace it with the following paragraph:

Recombinant plasmids. PSG5/LCoR, Flag-HDAC6/pcDNA3, HA HDAC3/pCDNA3.1, Flag-LCoR/pcDNA3.1 and LCoR derivatives mutagenized in the CtBP binding motifs, PLDLTVR **(SEQ ID NO: 15)** (LCoR a.a. 64-70; m1) and VLDLSTK **(SEQ ID NO: 16)** (LCoR a.a. 82-88; m2) and the double mutant (m1+2) have been described (Renaud JP et al.,

2000 *Cell & Mol. Life Sci* **57** 1748-69.). LCoR cDNAs mutated in the CtBP binding motifs were subcloned downstream of Flag in pCDNA3.1.

Please delete the paragraph on page 36, lines 10-25 and replace it with the following paragraph:

Our previous studies showed that LcoR interacts strongly and directly with CtBPs through tandem consensus motifs, and that the integrity of these motifs was essential for full corepression of hormone-dependent transcription. Colocalization of LCoR with CtBPs 1 and 2 tin MCF-7 cell nuclei was confirmed by immunocytochemical analyses (Figs. 8A and 8B). Both proteins were both broadly distributed in the nucleus and were also concentrated in discrete nuclear bodies. Given the functional interaction and the extensive overlap of CtBP and LCoR in the nucleus, we also investigated whether LCoR colocalized with CtBP-interacting proteins. CtBP-interacting protein (CtIP) was identified as a CtBP cofactor containing a PXLDLXXR **(SEQ ID NO: 22)** motif, whose association with CtBP was disrupted by ETA. Subsequently, CtIP was found to interact directly with the retinoblastoma gene produc). Remarkably, similar to results obtained with CtBP, CtIP and LCoR showed strongly overlapping patterns of expression in discrete nuclear bodies (Fig. 8C). We also observed a substantial colocalization of LCoR and Rb (Fig. 8D).

Please delete the paragraph on page 37, lines 5-25 and replace it with the following paragraph:

The association of LCoR with PcG complexes and associated proteins was further supported by coimmunoprecipitation experiments from MCF-7 cell extracts in which endogenous LCoR was detected in immunoprecipitates of endogenous proteins generated with antibodies directed against CtBP, CtIP, Rb and BMI1, but not with control antibody (Fig. 9). The coimmunoprecipitation of CtIP, and by extension Rb, and LCoR is remarkable given that CtIP and LCoR interact with CtBP through common PXLDLXXR **(SEQ ID NO: 22)** motifs. While repressors such as the Kruppel zinc finger protein Ikaros can interact simultaneously with CtBP and CtIP, no evidence was found for LCoR binding directly to CtIP or Rb in vitro

in GST pull- down experiments, indicating that their association in viva is indirect. Moreover, tagged wild-type LCoR or LCoR mutated in one of its two CtBP binding sites coimmunoprecipitated with endogenous CtBPs from extracts of MCF-7 cells, whereas no coimmunoprecipitation was observed in cells expressing an LCoR derivative (m1+2) mutated in both sites (Fig. 3, bottom panel). This is consistent with the observation that mutation of both CtBP binding sites of LCoR was required to abolish its interaction with CtBP *in vitro* (13). While the results show that LCoR binds directly to CtBPs through its cognate binding motifs *in vivo*, they also indicate that the two proteins do not also associate indirectly through stable interaction of LCoR with other components of PcG complexes.